sion; indeed, LPA induces the expression of various cytokines, including IL-6, IL-8, CXCL1, and CCL2 (e.g., Palmetshofer *et al.*, 1999; Fang *et al.*, 2004; Klemm *et al.*, 2007; C. Stortelers, unpublished results). That LPA is a key mediator of SMD activity is demonstrated by the failure of SMD to evoke biological effects in LPA receptor-negative cells (van Meeteren *et al.*, 2004). Thus, LPA rather than C1P is the likely trigger of the observed inflammatory response to *Loxosceles* SMD. Specific LPA antagonists could be useful tools for the treatment of bites by *Loxosceles* spiders.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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REFERENCES

Dragulev B, Bao Y, Ramos-Cerrillo B, Vazquez H, Olvera A, Stock R *et al.* (2006) Upregulation of IL-6, IL-8, CXCL1, and CXCL2 dominates gene expression in human fibroblast cells exposed to Loxosceles reclusa sphingomyelinase D: insights into spider venom dermonecrosis. *J Invest Dermatol.* doi:10.1038/sj.jid.5700644

Fang X, Yu S, Bast RC, Liu S, Xu HJ, Hu SX *et al.* (2004) Mechanisms for lysophosphatidic acid-induced cytokine production in ovarian cancer cells. *J Biol Chem* 279: 9653–61

Klemm S, Zimmermann S, Peschel C, Mak TW, Ruland J (2007) Bcl10 and Malt1 control lysophosphatidic acid-induced NF(kappa)B activation and cytokine production. *Proc Natl Acad Sci USA* 104:134–8

Lee S, Lynch KR (2005) Brown recluse spider (Loxosceles reclusa) venom phospholipase D (PLD) generates lysophosphatidic acid (LPA). *Biochem J* 391:317–23

Moolenaar WH, van Meeteren LA, Giepmans BN (2004) The ins and outs of lysophosphatidic acid signaling. *Bioessays* 26:870–81

Palmetshofer A, Robson SC, Nehls V (1999) Lysophosphatidic acid activates nuclear factor kappa B and induces proinflammatory gene expression in endothelial cells. *Thromb Haemost* 82:1532–7

Ramu Y, Xu Y, Lu Z (2006) Enzymatic activation of voltage-gated potassium channels. *Nature* 442:696–9

van Meeteren LA, Frederiks F, Giepmans BN, Pedrosa MF, Billington SJ, Jost BH *et al.* (2004) Spider and bacterial sphingomyelinases D target cellular lysophosphatidic acid receptors by hydrolyzing lysophosphatidylcholine. *J Biol Chem* 279:10833–6

Response to Moolenaar *et al.*

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TO THE EDITOR

We thank the authors for presenting these valuable comments and for giving us the opportunity to extend the discussion limited by the format of the paper. van Meeteren *et al.* (2004) confirmed that recombinant SMDs from Loxosceles laeta and *Corynebacterium pseudotuberculosis* possessed intrinsic lysophospholipase D activity to generate bioactive lysophosphatidic acid. They demonstrated that SMD did not activate mitogen-activated protein kinase (ERK1/2) in receptor-deficient B103 neuroblastoma cells, whereas both SMDs were activating mitogen-activated protein kinase when the same cells expressed lysophosphatidic $acid_1$ receptors. They also showed that bacterial and *Loxosceles* SMDs triggered receptor internalization in HEK293 cells only when preincubated with albumin-LPC. Lee and Lynch (2005) extended the findings by demonstrating that recombinant *L. reclusa* SMD hydrolzses various lysophospholipids and identified specific histidine residues that are essential for the enzyme activity.

Pettus *et al.* (2003, 2004) in two consequential investigations provided evidence that ceramide-1-phosphate (C1P) interacts directly with cytosolic phospholipase A_2 ($cPLA_2$) acting as an activator of $cPLA_2$ and subsequent inflammatory response. They determined that in A549 lung adenocarcinoma cells, natural and endogenous (produced by SMD) C1Ps were potent and specific inducers of arachidonic acid and prostanoid synthesis. The treatment of A549 cells with SMD resulted in a threefold increase in arachidonic acid release. Using RNA-mediated interference technology, Pettus *et al.* confirmed in A549 and in J774.1 microphages that $cPLA_2$ was downstream of C1P. It was also found that in A549 cells, C1P caused translocation of $cPLA_2$ to membranes. Their *in vitro* binding studies disclosed that C1P directly binds and activate with full-length $cPLA_2$. Other publications regarding biological activities of C1P are reviewed in details by Gomez-Munoz (2004).

The focus of our investigation was the expression pattern of human fibroblasts treated with recombinant SMD to gain insight into cellular mechanisms of loxoscelism pathology. We observed a dose- and time-dependent upregulation of several cytokines (data not shown). The pattern of continuous increase was present up to 18 hours of treatment (data not shown). Although we recognize the evidence that lysophospholipase D activity of SMD is obviously an important factor in loxoscelism, we are not convinced that the sphingomyelin–ceramide pathway involvement in the *L. reclusa* pathology can be ignored. More than one mechanism of action is also possible in this pathology and further investigation would benefit the understanding of the complex immunological response following spider envenomation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Abbreviation: EP, epidermis

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REFERENCES

Gomez-Munoz A (2004) Ceramide-1-phosphate: a novel regulator of cell activation. *FEBS Lett* 562:5–10

Lee S, Lynch KR (2005) Brown recluse spider (*Loxosceles reclusa*) venom phospholipase D (PLD) generates lysophosphatidic acid (LPA). *Biochem J* 391:317–23

Pettus BJ, Bielawska A, Spiegel S, Roddy P, Hannun YA, Chalfant CE (2003) Ceramide kinase mediates cytokine- and calcium ionophore-induced arachidonic acid release. *J Biol Chem* 278:38206–13

Pettus BJ, Bielawska A, Subramanian P, Wijesinghe DS, Maceyka M, Leslie CC *et al.* (2004) Ceramide 1-phosphate is a direct activator of cytosolic phospholipase A2. *J Biol Chem* 27:11320–6

van Meeteren LA, Frederiks F, Giepmans BN, Pedrosa MF, Billington SJ, Jost BH *et al.* (2004) Sphingomyelinases D target cellular lysophosphatidic acid receptors by hydrolyzing lysophosphatidylcholine. *J Biol Chem* 27:10833–6

Epidermal Transglutaminase Deposits in Perilesional and Uninvolved Skin in Patients with Dermatitis Herpetiformis

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TO THE EDITOR

Dermatitis herpetiformis (DH) is a disease precipitated by ingestion of gluten and characterized by IgA deposits in the dermal papillae (Nicolas *et al.*, 2003). Like celiac disease, DH is one presentation of gluten-sensitive enteropathy (Zone, 2005). In susceptible individuals, ingestion of gluten leads to elaboration of antibodies against gliadin and transglutaminase 2 (TG2) (Oxentenko and Murray, 2003; Alaedini and Green, 2005; Zone, 2005). TG2, one of nine homologous transglutaminases, is an autoantigen in celiac disease (Dieterich *et al.*, 1997; Lorand and Graham, 2003). Serum antibodies against TG2 can be sensitive and specific for DH as well as celiac disease (Dieterich *et al.*, 1999).

Sardy *et al.* (2002) hypothesized that an autoantigen related to TG2 could explain the presentation of DH and reported that epidermal transglutaminase (TG3) deposits with IgA in perilesional biopsies in patients with DH. IgA deposits can occur in regions that are not clinically involved (Fry *et al.*, 1978; Zone *et al.*, 1996). To confirm the involvement of TG3 in DH and to determine whether TG3 deposits are restricted to perilesional skin, we have raised a new goat antibody against human TG3.

In a series of nine DH patients with active disease, we compared direct immunofluorescence (DIF) for IgA and TG3 in perilesional and uninvolved skin. We also compared serology for TG2 and TG3 and results from DIF for IgA and TG3 in the context of a gluten-free diet (GFD). The protocol was approved by our Institutional Review Board, patients consented in writing, and we adhered to the Declaration of Helsinki Principles. All patients had a diagnosis of DH established by skin biopsy showing granular IgA and had active disease. Adherence to GFD was reported by patients as strict or poor.

Serum was analyzed for IgA antibodies against TG2, TG3, and endomysium endomysial antibody (EMA). Semiquantitative detection of anti-TG2 and -TG3 were performed using ELISA (INOVA Diagnostics, San Diego, CA, and Immunodiagnostik AG, Bensheim, Germany). EMA were detected using indirect immunofluorescence on monkey esophagus (University of California at Davis) (Unsworth, 1996). Patient age, sex, family history, adherence to GFD, dapsone use, and serologies for antibodies against TG2, TG3, and endomysium are represented in Table 1. Five patients reported strict adherence to a GFD. Among patients adhering to a strict GFD, four of five (80%) were negative by IgA ELISA for anti-TG2 antibodies and EMA. All four patients reporting poor GFD adherence had anti-TG2 antibodies and two of four were positive by EMA. All five patients in the strict GFD group were seronegative for anti-TG3 antibodies and all four patients in the poor GFD group were positive. The two patients with negative EMA had the lowest values for anti-TG2.

Polyclonal TG3 antibody

Purified TG3 expressed from a baculovirus system was used to produce polyclonal antibodies in goats (Ahvazi and Steinert, 2003). Purified TG3 was a kind gift from Dr Bijan Ahazi. To confirm reactivity and specificity, Western blots were performed using the baculovirus-produced TG3 as well as separately cloned TG2 and TG3. Complementary DNAs for TG3 and TG2 were isolated by RT-PCR from human keratinocytes using primers published by Sardy *et al.*, and were sequence confirmed. Complementary DNA was cloned in the Invitrogen pSecTag2/Hygro B vector, lysates were purified using a Ni^{2+} column (Invitrogen, Carlsbad, CA, USA), electrophoresed, and transferred to nitrocellulose blots. IgG was prepared using a Protein G column. Western blots confirmed a high titer against both TG3 clones. Specificity was demonstrated by lack of reactivity against non-transfected human embryonic kidney lysate, recombinant TG2 (Figure 1), and serum and secretory (colostral) IgA (not shown). Further specificity was established by lack of reactivity on IIF when reacted with skin from three patients with linear IgA bullous dermatosis (Figure 2e and f), two with Henoch–

Abbreviations: DIF, direct immunofluorescence; DH, dermatitis herpetiformis; EMA, endomysial antibody; GFD, gluten-free diet; TG2, transglutaminase 2 or tissue transglutaminase; TG3, transglutaminase 3 or epidermal transglutaminase